

Fig. 1

Fig 1 E - cont

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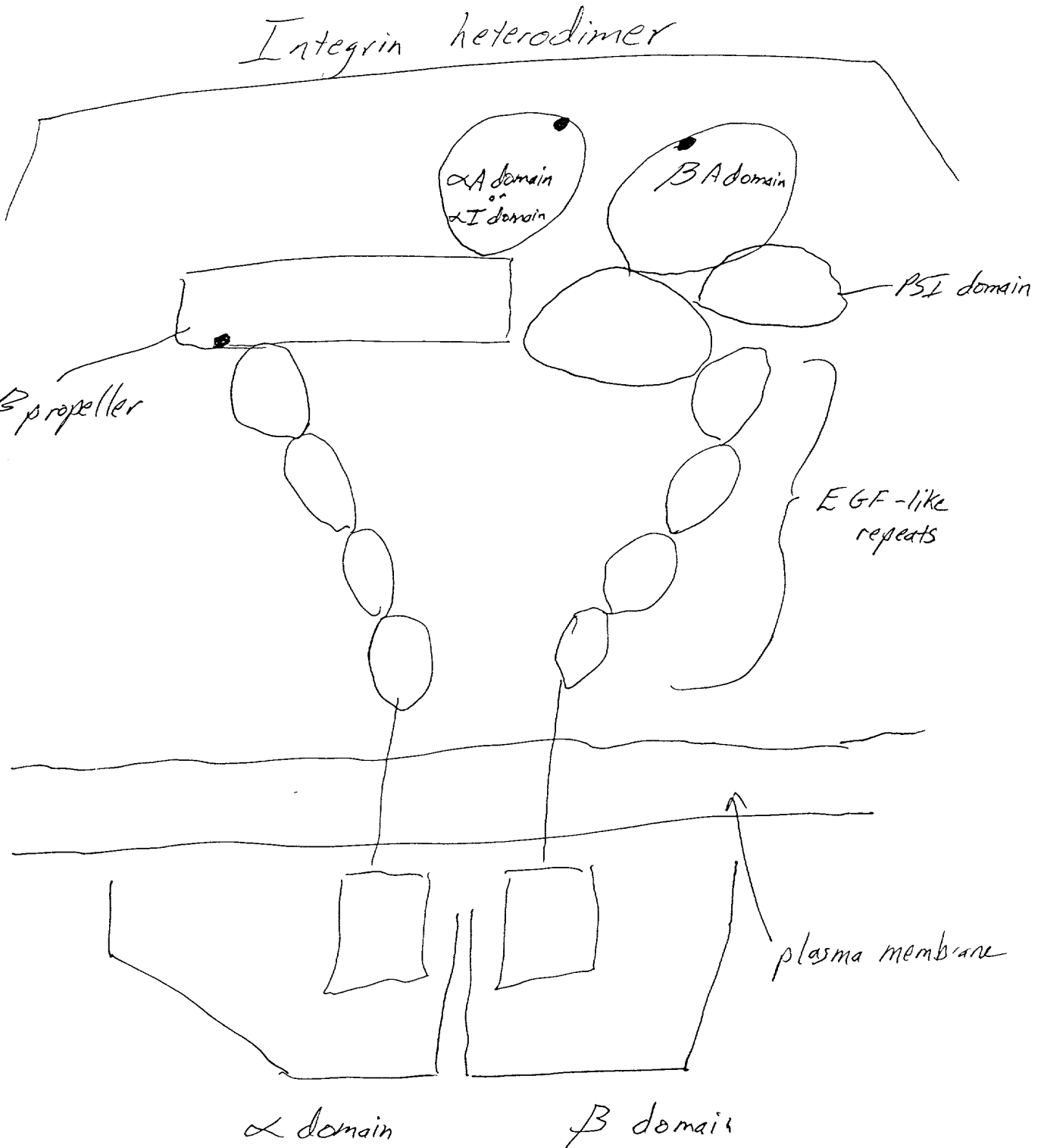


Fig 1F

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05040" 040500

BASE COUNT 1095 a 1271 c 1289 g 1085 t  
 ORIGIN 1 bp upstream of EcoRI site.

Fig 1 F

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Fig/F

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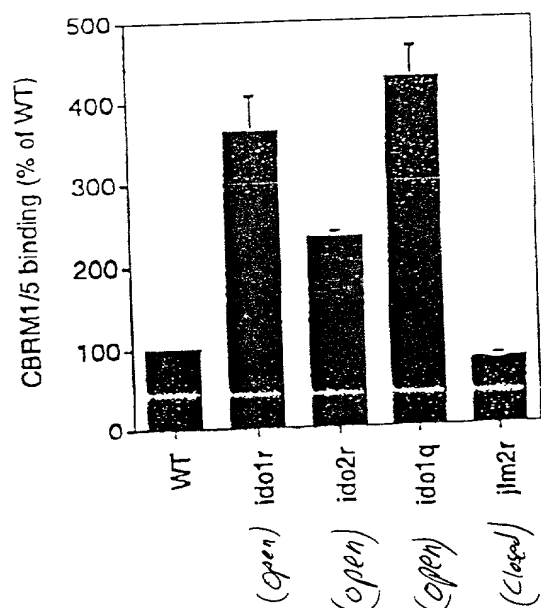
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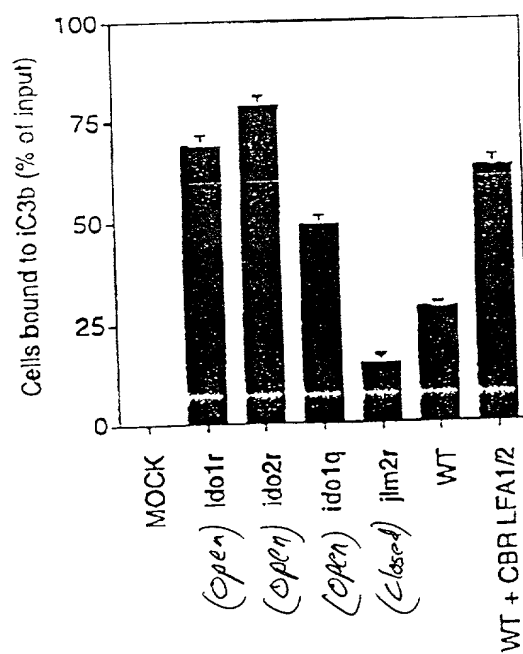
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Fig 2

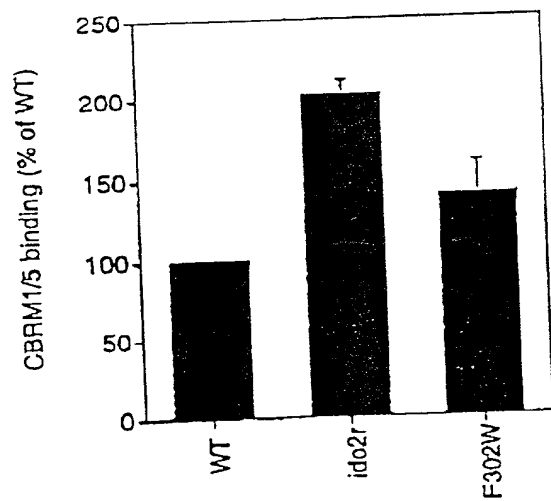
A



B



C



D

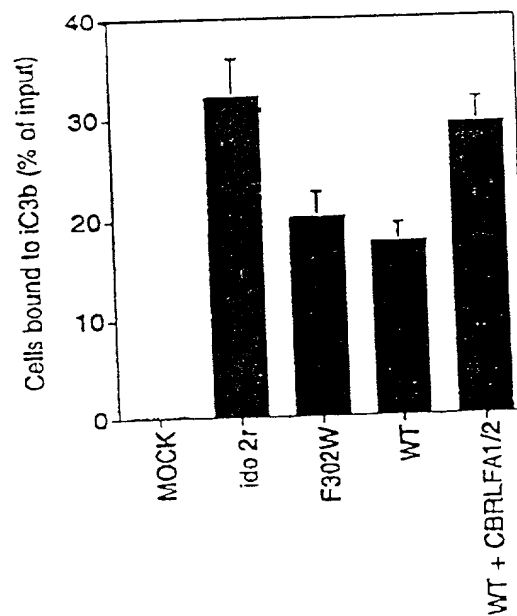


Fig. 3

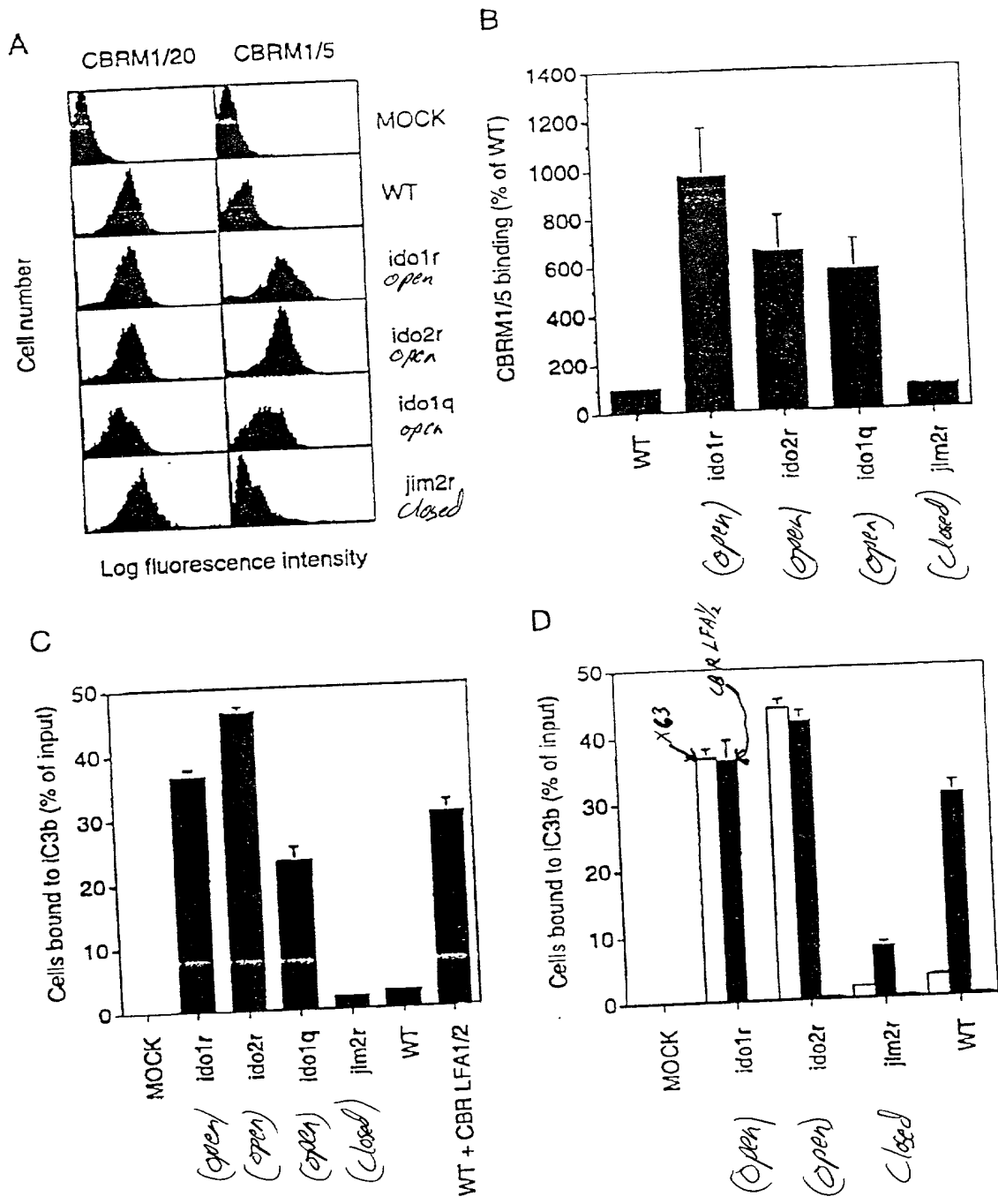
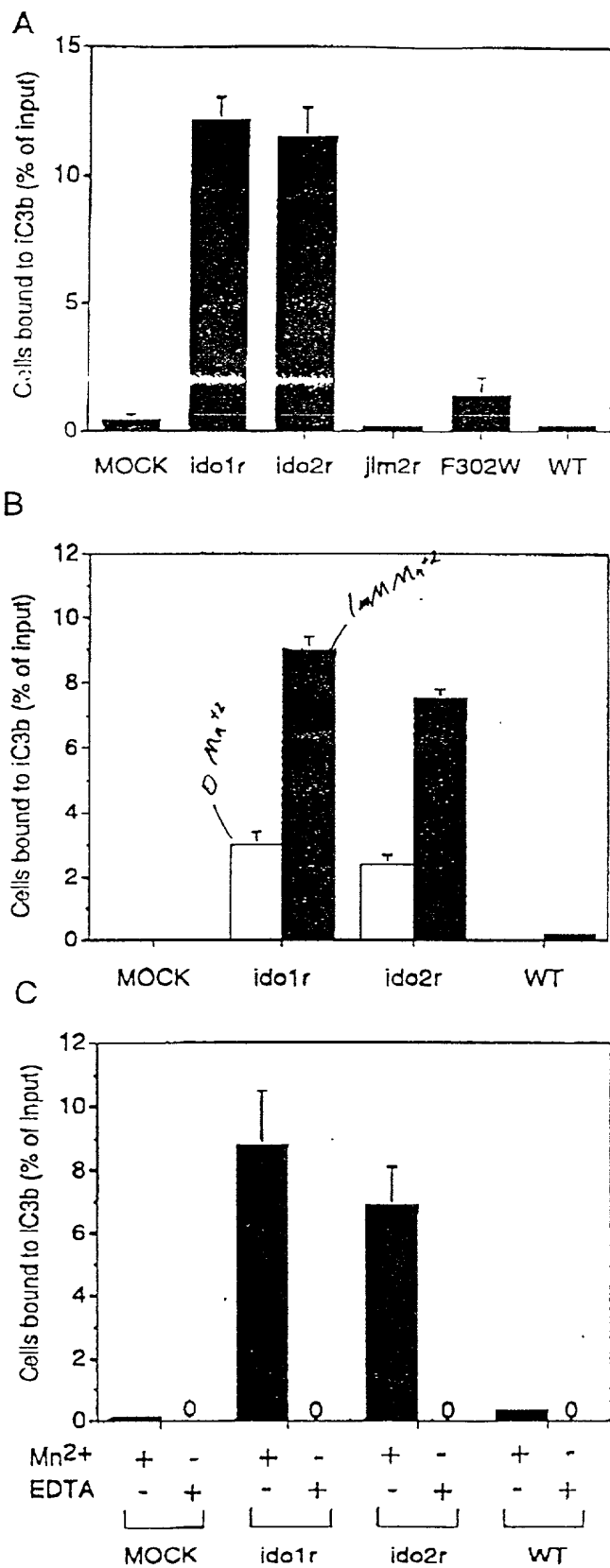


Fig 4



06020-18420660



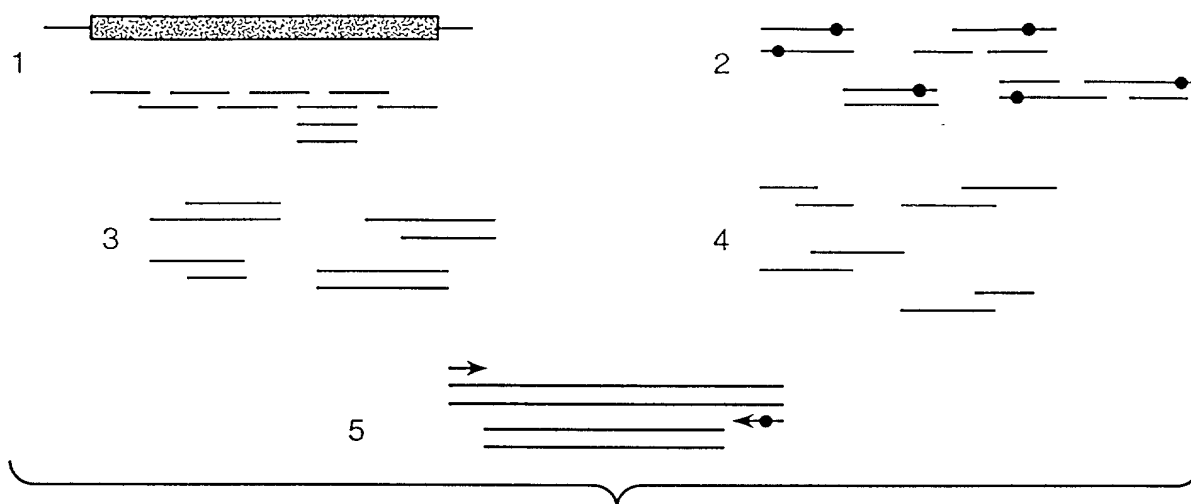


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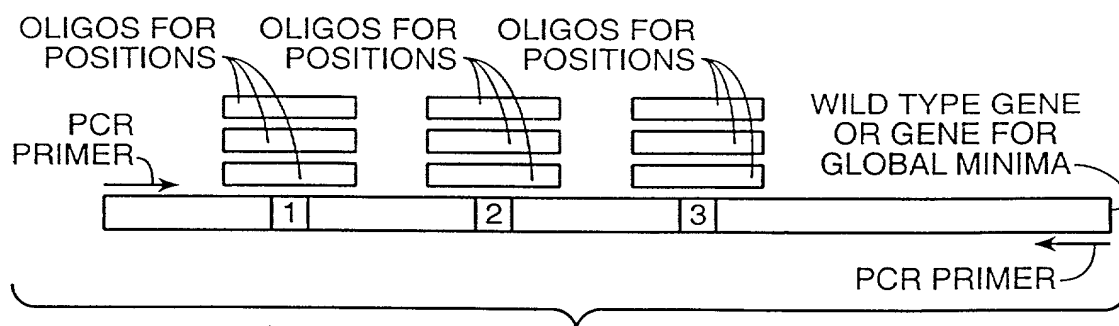
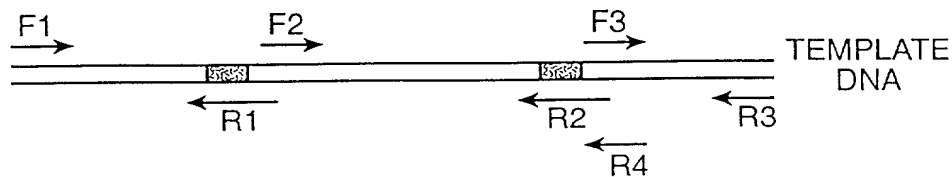


Fig 6

BLACK BOX =  
REGION TO  
BE MUTATED



STEP 1: SET UP 3 PCR REACTIONS:

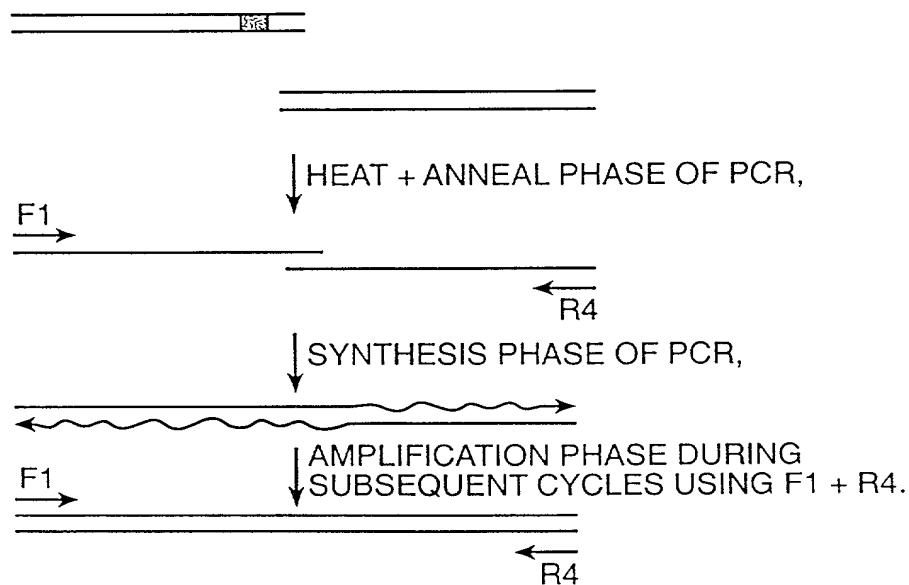
PRODUCTS:

TUBE 1:

TUBE 2:


TUBE 3:

STEP 2: SET UP PCR REACTION WITH PRODUCTS OF TUBE 1 +  
PRODUCTS TUBE 2 + F1 + R4.




STEP 3: REPEAT STEP 2 USING PRODUCT FROM STEP 2 + PRODUCT  
FROM STEP 1, TUBE 3 + PRIMERS F1 + R3.

FIG. 7

TUBE 1: 

TUBE 2:

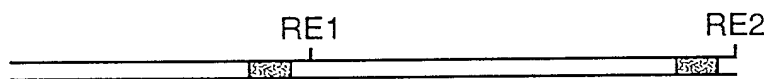


RE1 RE2

TUBE 3: RE2

STEP 2: DIGEST PRODUCTS FROM STEP 1 WITH SUITABLE RESTRICTION ENDONUCLEASES.

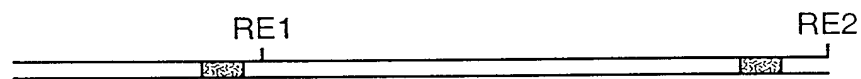
**STEP 3:** LIGATE DIGESTED PRODUCT FROM STEP 2, TUBE 2 WITH DIGESTED PRODUCT FROM STEP 2, TUBE 1.



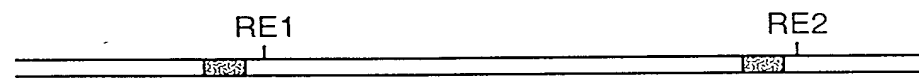
**STEP 4: AMPLIFY VIA PCR LIGATED PRODUCTS OF STEP 3 WITH F1 + R4.**



STEP 5: DIGEST AMPLIFIED PRODUCT OF STEP 4  
WITH RESTRICTION ENDONUCLEASE #2.



**STEP 6:** LIGATE PRODUCT FROM STEP 5 WITH  
PRODUCT FROM STEP 2, TUBE 3.



**STEP 7:** AMPLIFY PRODUCT FROM STEP 6 WITH F1 + R3.

Fig 8  
DIAGRAM 3



Fig. 9